Figure S1

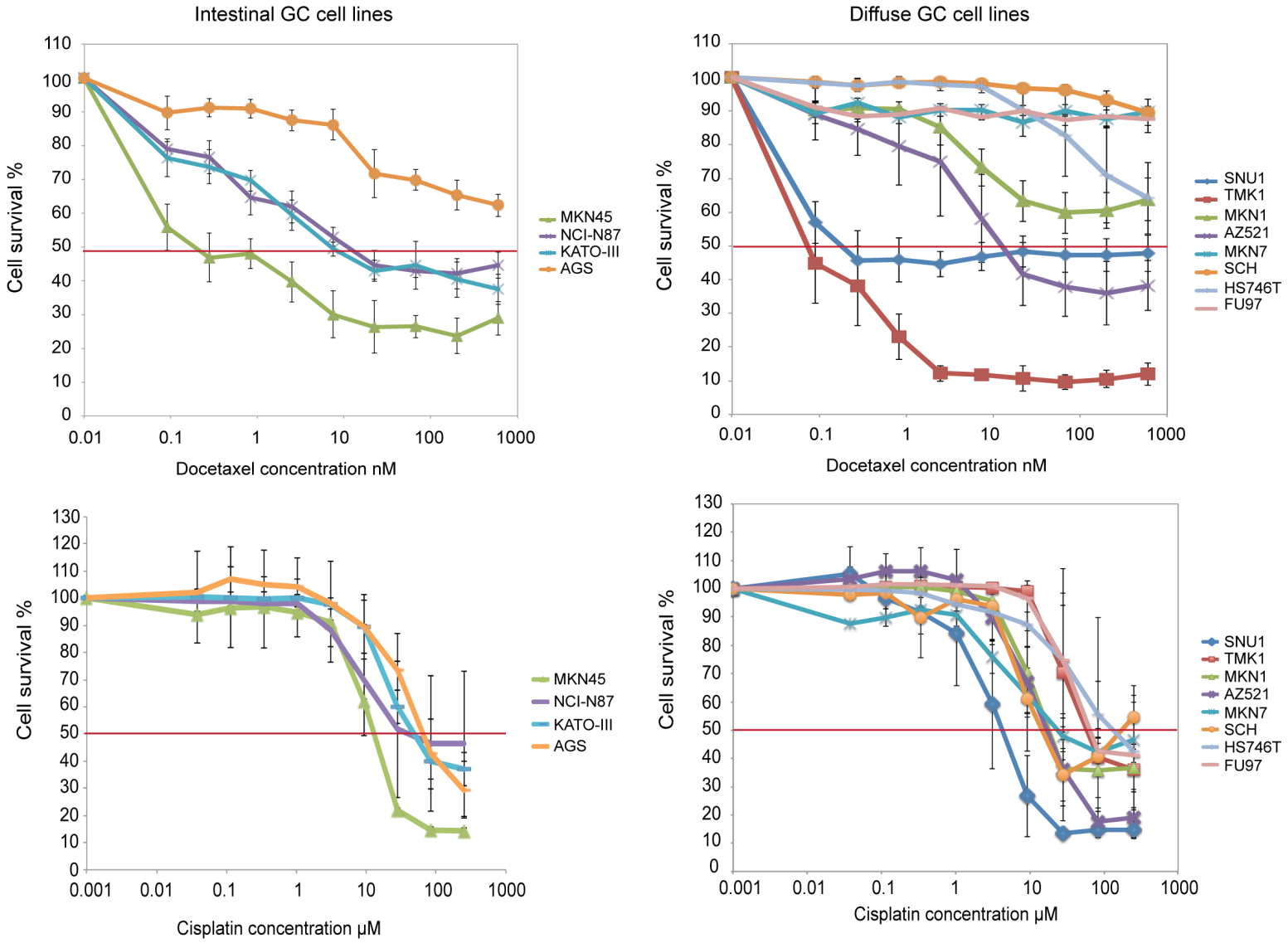

Intestinal GC cell lines
Cell survival %
Docetaxel concentration nM
MKN45
NCI-N87
KATO-III
AGS
Diffuse GC cell lines
SNU1
TMK1
MKN1
AZ521
MKN7
SCH
HS746T
FU97
Cisplatin concentration µM

Figure S1. Cell viability assay after docetaxel or cisplatin treatment in INT-GC and DIF-GC cell lines. INT-GC and DIF-GC cell lines were treated with increasing concentrations of docetaxel for 72 hours. Cell survival was assessed with Sulforhodamine B (SRB) assay and IC50 is referred as the drug concentration at which 50% of cell death is achieved. X-axis shows drug concentrations expressed in nM for docetaxel treatment and μM for cisplatin treatment; y-axis reports percentage of cell survival, calculated for each concentration of the drug with the following formula: (treatment/control) x 100. SD showed as error bar. Three of the four (75%) INT-GC cell lines (MKN45, NCI-N87 and KATO-III) reached an IC50 value (IC50 from 0.2nM to 11nM) and only the INT-GC AGS cell line showed resistance (IC50 not reached). Conversely, only three of the eight (37%) DIF-GC cells (TMK1, SNU1 and AZ521) showed sensitivity to DTX, (IC50 from 0.08nM to 13nM) and five out of eight DIF-GC cell lines (FU97, HS746T, SCH, MKN7 and MKN1) exhibited resistance (IC50 not reached). Three of the five DTX-resistant DIF-GC cells (MKN-1, SCH and MKN7) showed sensitivity towards cisplatin, ruling out an unspecific drug resistance.

Figure S2

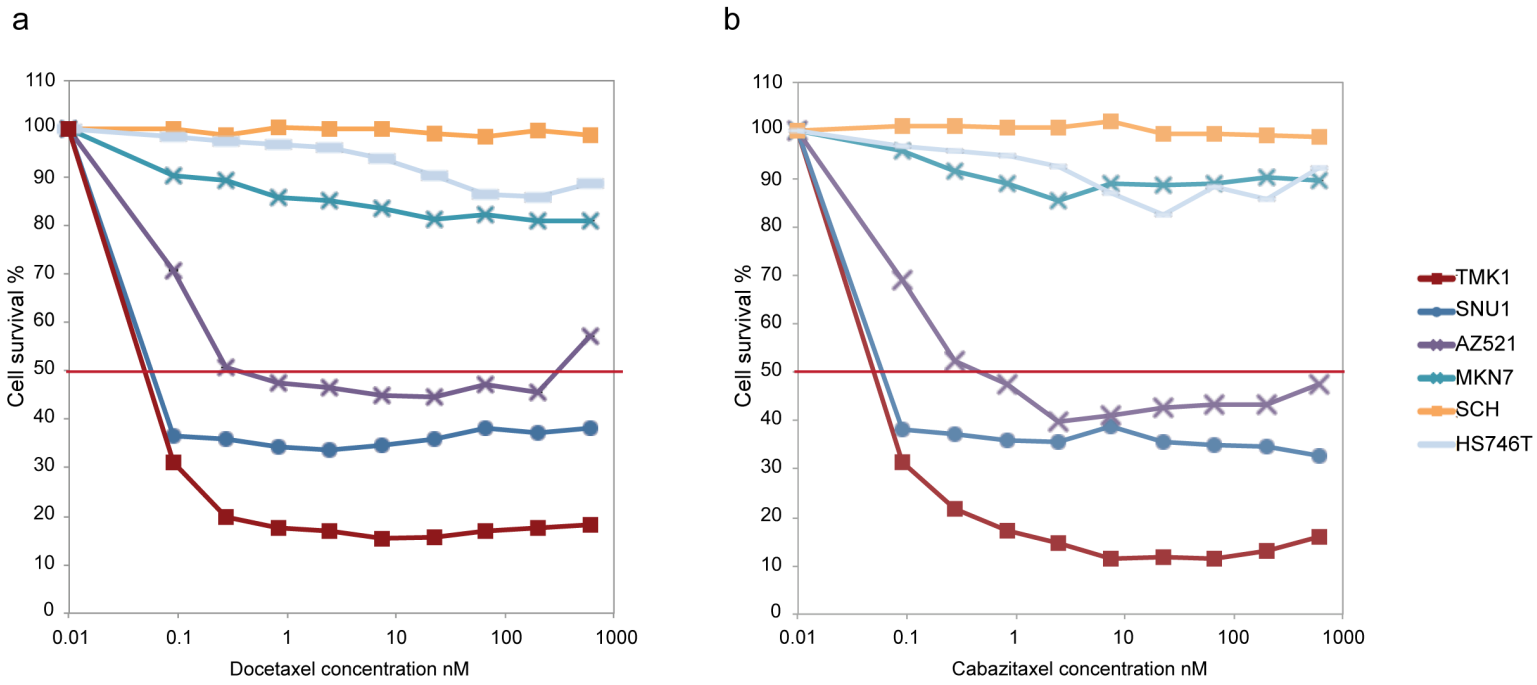
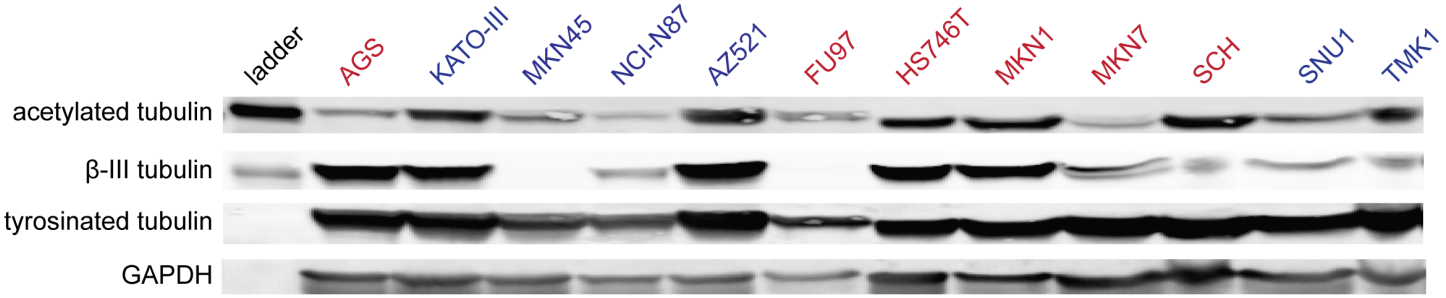

a
b
Cell survival %
Docetaxel concentration nM
Cabazitaxel concentration nM
0.01
0.1
1
10
100
1000
0
10
20
30
40
50
60
70
80
90
100
110
TMK1
SNU1
AZ521
MKN7
SCH
HS746T

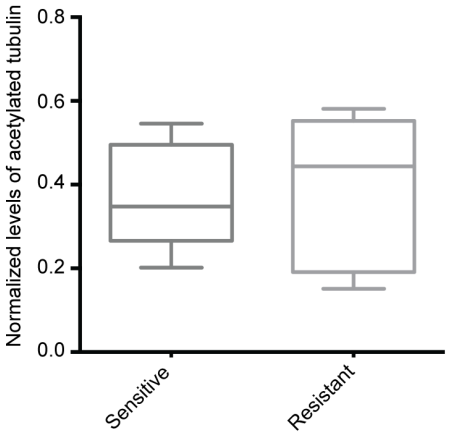
Figure S2. Cell viability assay after docetaxel (a) or cabazitaxel (b) treatment in DIF-GC cell lines. Cells were treated with increasing concentrations of docetaxel or cabazitaxel for 72 hours. Cell survival was assessed with Sulforhodamine B (SRB) assay and IC50 is referred as the drug concentration at which 50% of cell death is achieved (red orizontal line). X-axis shows drug concentrations expressed in nM for docetaxel and cabazitaxel treatment; y-axis reports percentage of cell survival, calculated for each concentration of the drug with the following formula: (treatment/control) x 100.

Figure S3

a



b



c

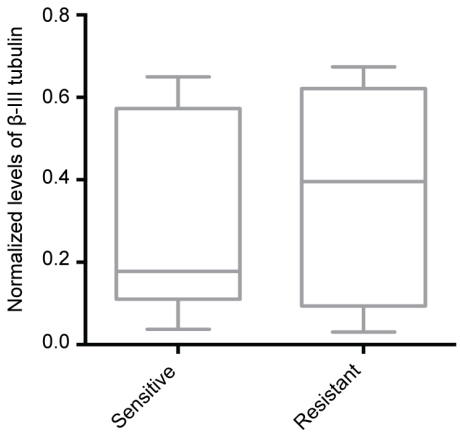


Figure S3. Protein expression profile of acetylated tubulin, β-III tubulin isotype and tyrosinated tubulin in GC cell lines. **a**. 50 μg of total protein extract were separated through electrophoresis; PVDF membranes were stained for β-III tubulin, acetylated tubulin and tyrosinated tubulin; GAPDH was used as loading control. **b**. Box plots displaying normalized expression levels of acetylated tubulin (b) and β-III tubulin (c) in DTX-sensitive versus DTX-resistant GC cell lines.

Figure S4

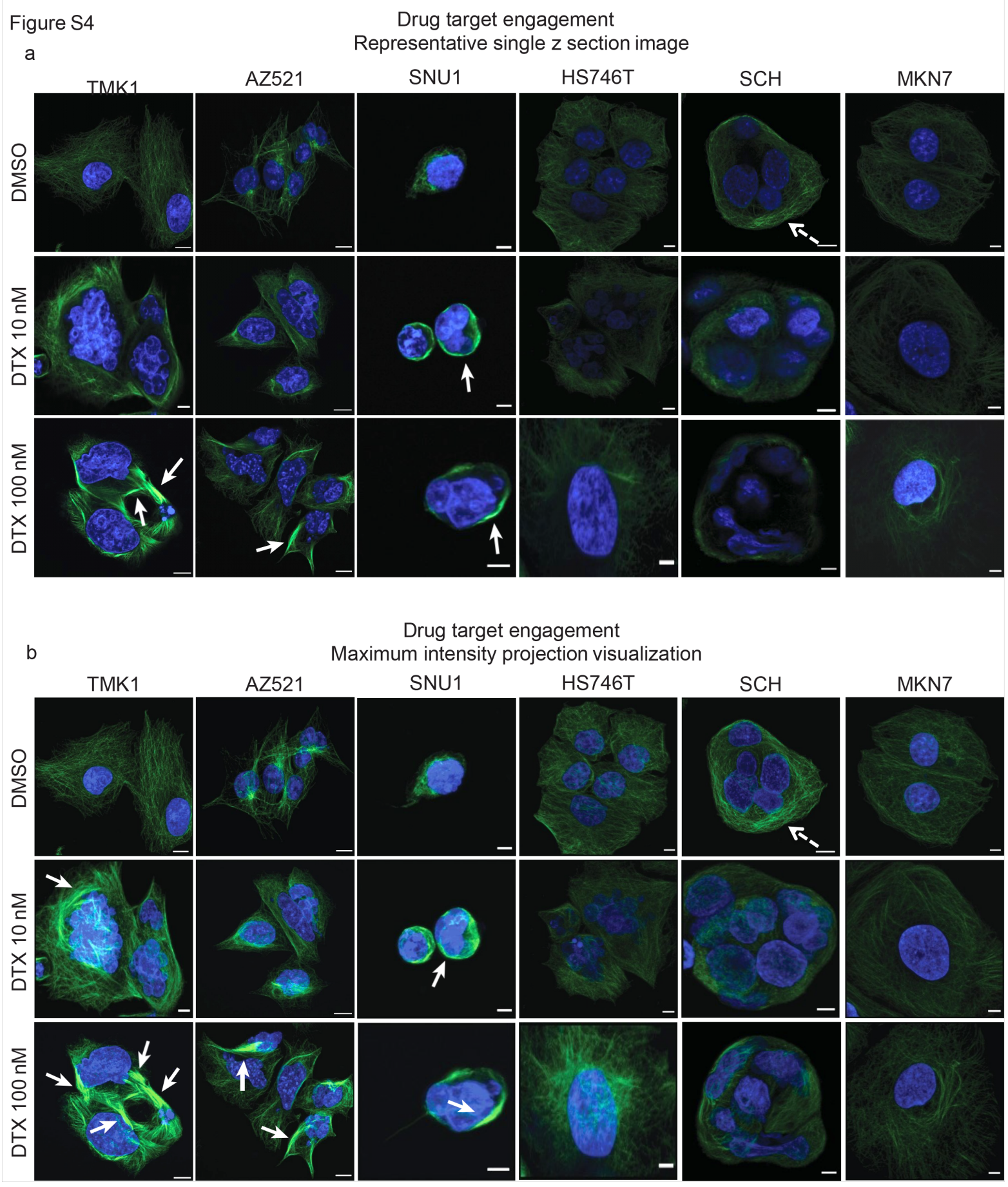

a
Drug target engagement
Representative single z section image
TMK1
AZ521
SNU1
HS746T
SCH
MKN7
DMSO
DTX 10 nM
DTX 100 nM
b
Drug target engagement
Maximum intensity projection visualization
TMK1
AZ521
SNU1
HS746T
SCH
MKN7
DMSO
DTX 10 nM
DTX 100 nM

Figure S4. Impaired drug-target engagement in resistant GC cell lines. Tubulin immunofluorescence in GC cells treated with docetaxel (DTX) (10 nM and 100 nM) or DMSO for 24 hours. Cells were fixed and immunostained for a-tubulin (green) and DAPI for the nucleus (blue). Cells were imaged by confocal microscopy acquiring several z-sections for each cell covering the entire cell volume. Representative cell images are shown either as single plane z-sections (**a**) or as maximum intensity projection (MIP) where all z-sections are collapsed in a 2D image thus, representing all MTs throughout the cell thickness (**b**). Drug target engagement (DTE) is evidenced by microtubule bundling (white arrows). Thick and bright microtubule bundles can be detected at single z-section level in the DTX-sensitive cells (TMK1, SNU1 and AZ521); this pattern is more clearly discernible in the MIP images. No evidence of microtubule bundling can be seen in the DTX-resistant cells (HS746T, SCH and MKN7). Slight MT network reorganization around the edge of the cell can be seen in MIP images of DTX-resistant cells (SCH, dashed arrow), due to post-acquisition MIP image rendering. This effect is independent of DTX treatment and is not sign of DTE, as no evidence of MT-bundling can be detected in single z-section (dashed arrow). Scale bar: 5 μm.

Figure S5

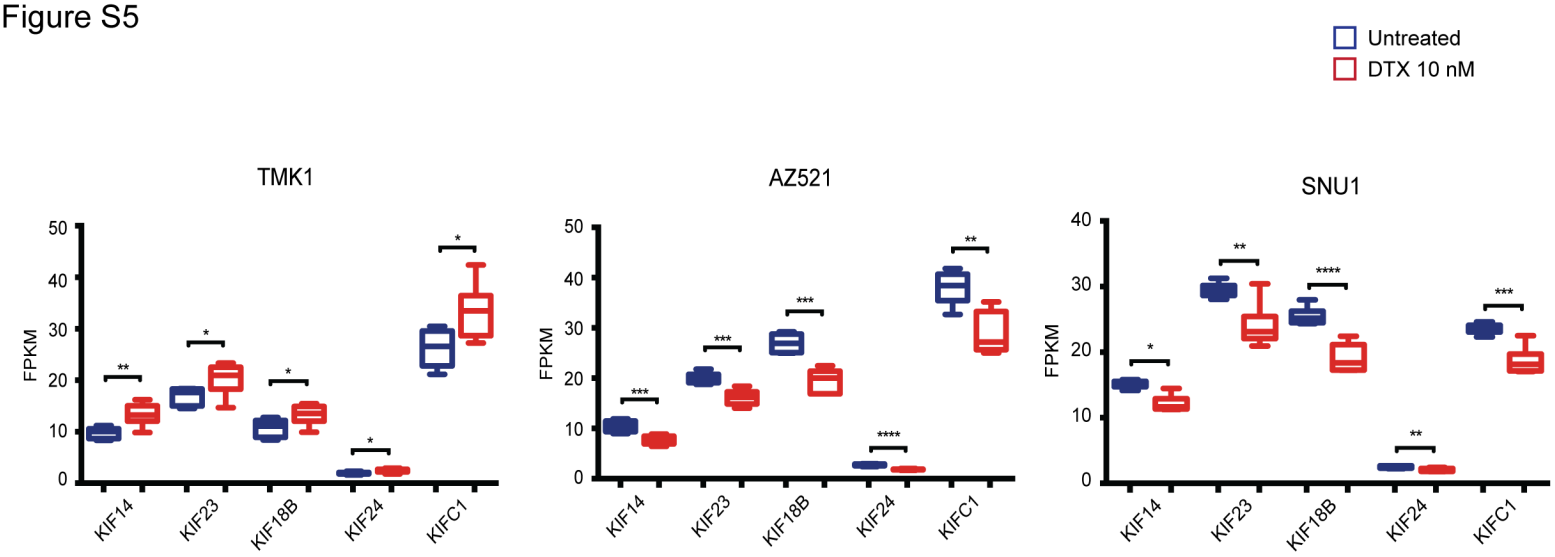

Untreated
DTX 10 nM
TMK1
AZ521
SNU1
FPKM
KIF14
KIF23
KIF18B
KIF24
KIFC1

Figure S5. Gene expression pattern of different KIF family members and their modulation by docetaxel (DTX) treatment. Blue, baseline; red, DTX treatment (10 nM, 24 hr). Gene expression is expressed in FPKM, before (blue) and after (red) docetaxel treatment. Student t-test was used to calculate significance.

Figure S6

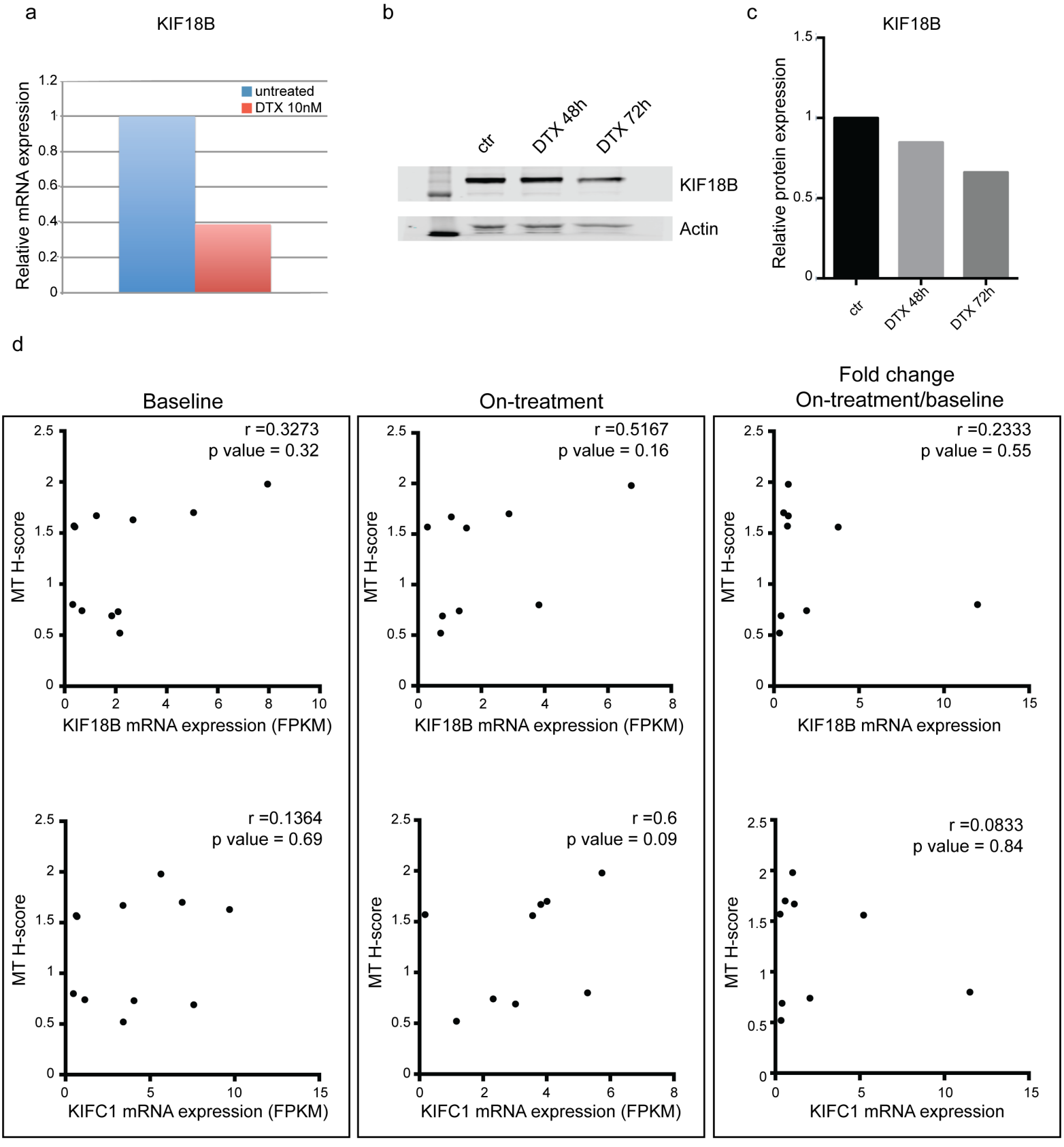

a
KIF18B
Relative mRNA expression
untreated
DTX 10nM
b
ctr
DTX 48h
DTX 72h
KIF18B
Actin
c
KIF18B
Relative protein expression
ctr
DTX 48h
DTX 72h
d
Baseline
On-treatment
Fold change
On-treatment/baseline
MT H-score
r =0.3273
p value = 0.32
KIF18B mRNA expression (FPKM)
r =0.5167
p value = 0.16
KIF18B mRNA expression (FPKM)
r =0.2333
p value = 0.55
KIF18B mRNA expression
r =0.1364
p value = 0.69
KIFC1 mRNA expression (FPKM)
r =0.6
p value = 0.09
KIFC1 mRNA expression (FPKM)
r =0.0833
p value = 0.84
KIFC1 mRNA expression

Figure S6. mRNA expression levels by RT-qPCR (**a**) and protein expression levels by Western blot (**b and c**) of KIF-18B in the docetaxel-sensitive AZ521 cell line. RNA and protein were collected before and after treatment with docetaxel (DTX). d. Correlation between MT H-score in cabazitaxel-treated GC patients biopsies (n=11) and KIFs mRNA expression at baseline, on-treatment and as fold change on-treatment/baseline. Spearman correlation coefficients and p-values are shown.

Figure S7

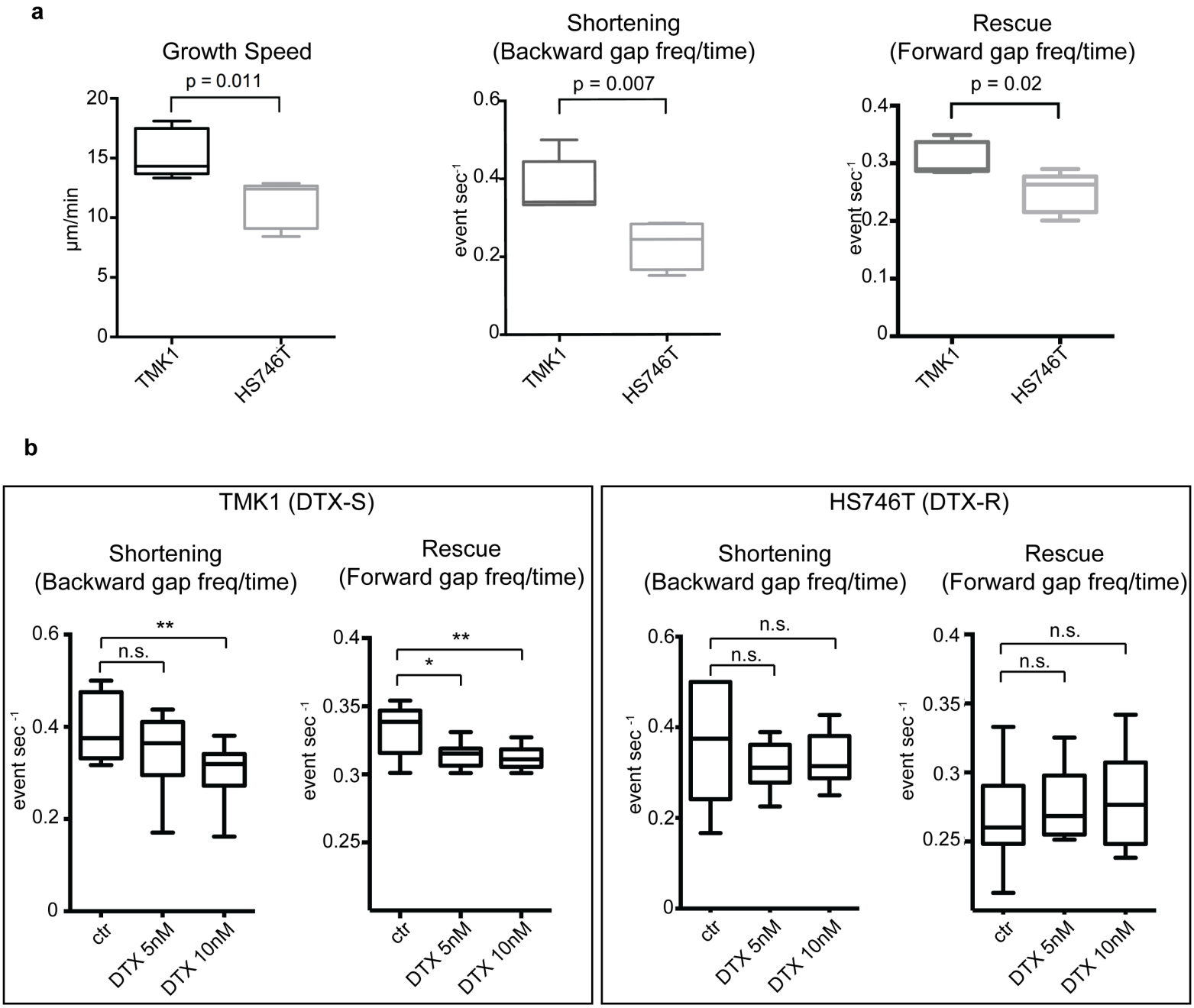
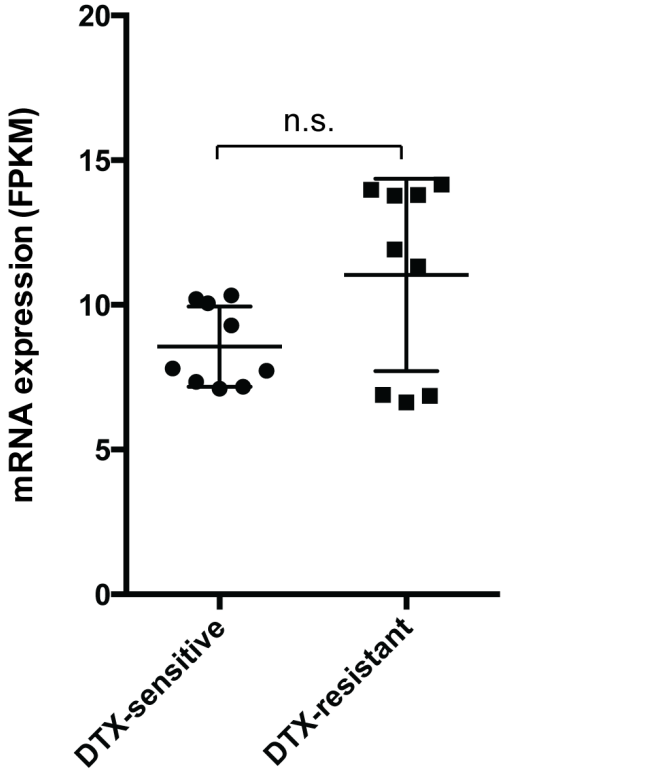

a
Growth Speed
p = 0.011
μm/min
20
15
10
5
0
TMK1
HS746T
Shortening
(Backward gap freq/time)
p = 0.007
event sec-1
0.6
0.4
0.2
0
TMK1
HS746T
Rescue
(Forward gap freq/time)
p = 0.02
event sec-1
0.4
0.3
0.2
0.1
0
TMK1
HS746T
b
TMK1 (DTX-S)
Shortening
(Backward gap freq/time)
Rescue
(Forward gap freq/time)
event sec -1
**
n.s.
*
ctr
DTX 5nM
DTX 10nM
HS746T (DTX-R)
Shortening
(Backward gap freq/time)
Rescue
(Forward gap freq/time)
n.s.
n.s.
ctr
DTX 5nM
DTX 10nM

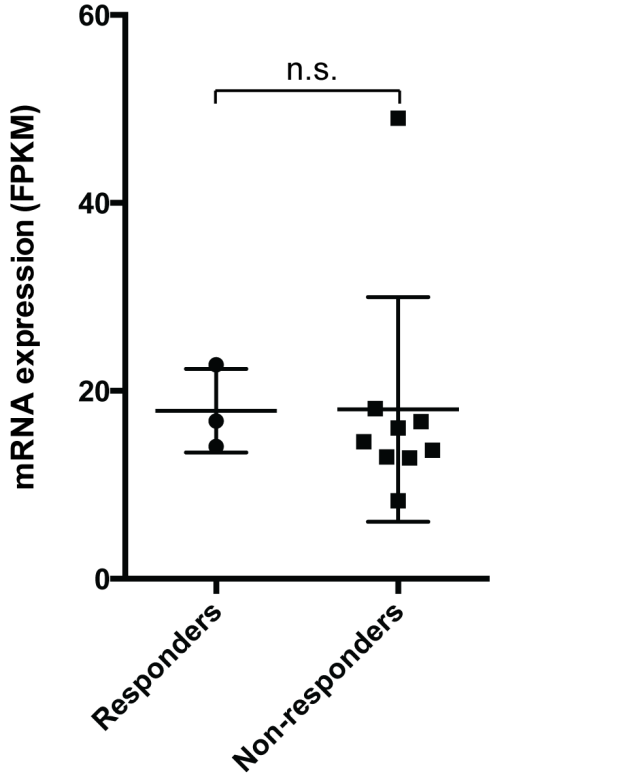
Figure S7. Computational analysis of MT dynamic parameters by direct detection and tracking of GFP-EB1 comets and by inference of pauses and shrinkage events, using the MATLAB®-based plusTipTracker algorithm. **a**. Graphic display of the following baseline microtubule dynamics parameters in TMK1 and HS746T cell lines: Growth Speed (μm x min-1; directly measured) and the computationally inferred Shortening (backward gap frequency) and Repolymerization/rescue (forward gap frequency) events. **b**. Graphic display of the changes in microtubule dynamicity (shortening and repolymerization/rescue events upon 1 h treatment with docetaxel (DTX) in TMK1 (sensitive, DTX-S) and HS746T (resistant,DTX-R) cells. Mann-Withney test was used for statistical analysis. * = $p < 0.05$; ** = $p < 0.01$. n.s. = not significant.

Figure S8

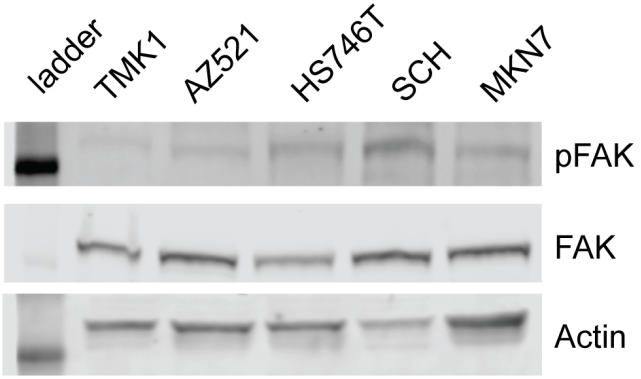
a



b



c



d

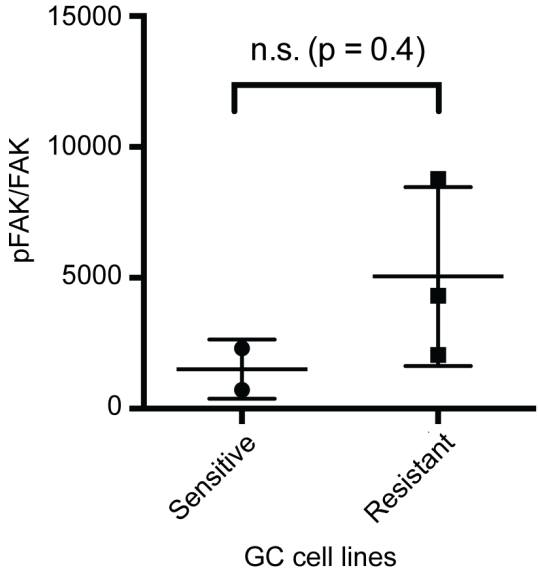
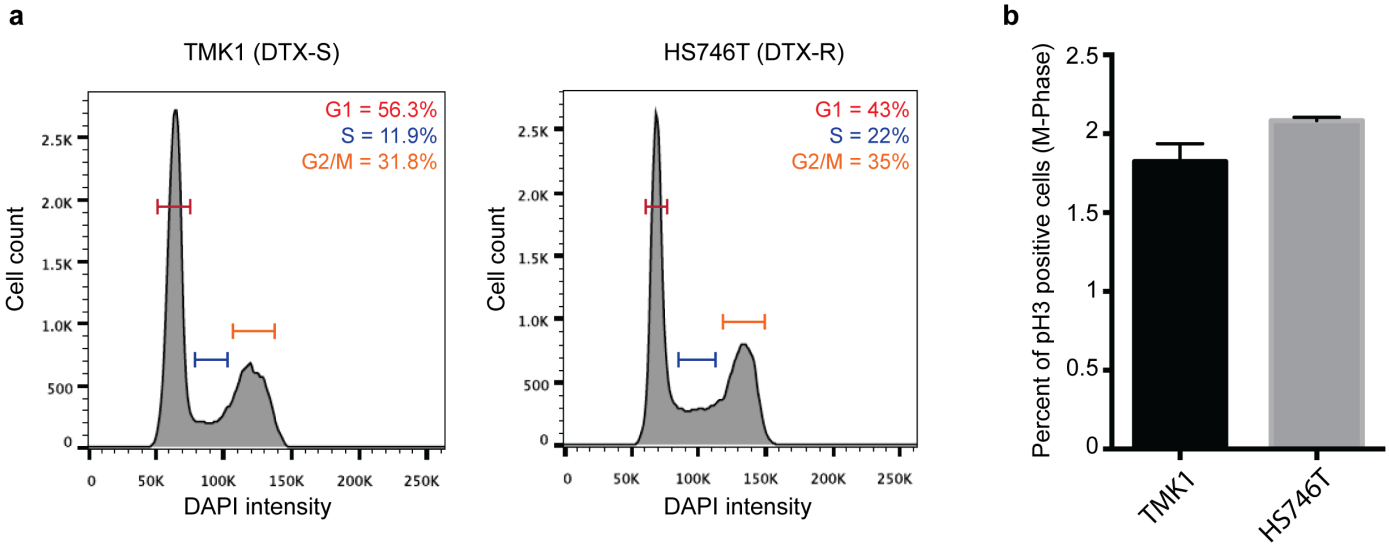
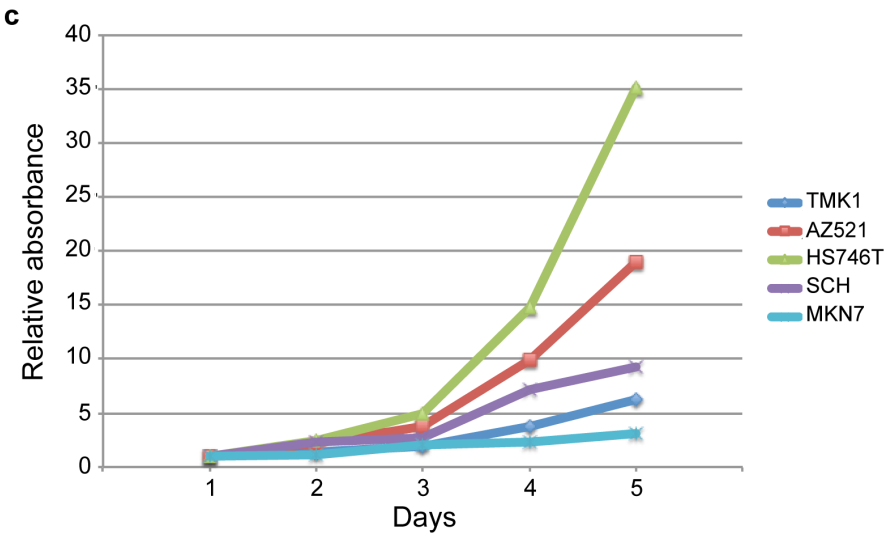


Figure S8. Focal Adhesion Kinase (FAK) expression and activation in GC cell lines and GC patient samples. Baseline FAK mRNA expression levels in DTX-sensitive (TMK1, AZ521 and SNU1) and DTX-resistant (HS746T, SCH and MKN7) cell lines (**a**) and in gastric cancer patients receiving cabazitaxel treatment (**b**); FPKM values of three biological replicates are displayed for each cell line. c. Phosphorylation of FAK (pFAK) at the Tyr397 residue was measured by western blot to quantify FAK activation in DTX-sensitive (TMK1 and AZ521) and DTX-resistant (HS746T, SCH and MKN7) cell lines. Graphic display of pFAK/FAK ratio in GC cell lines; densitometric analysis of pFAKand FAK of figure S8c was performed in each cell line. Values are grouped based in DTX-sensitivity as determined in Figure 1b.

Figure S9





d

	Doubling time (h)
TMK1	33.7
AZ521	22.3
HS746T	18.3
SCH	31.5
MKN7	58.7

Figure S9. Baseline cell cycle distribution analysis and doubling time quantification in GC cell lines. **a**. Quantification of the cell cycle distribution in DTX-sensitive (DTX-S) TMK1 and DTX-resistant (DTX-R) HS746T GC cells is shown; cells were fixed and stained with DAPI, and the cell cycle phase distribution was evaluated by flow cytometry. Histograms show number of cells in different phases (G1, S and G2/M). **b**. Quantification of cells in M-phase; cells were fixed and stained for phospho Histone 3 (pH3, marker of mitosis) and DAPI; fluorescence intensity was analyzed by flow cytometry. Percent of total cells positive for pH3 is shown. **c**. Assessment of GC cell growth rates by crystal violet protein staining. Cells were plated at day 0 and fixed every day for 4 consecutive days. All values are normalized to day 1. **d**. Doubling time in hours (h) is reported for each cell line.

Figure S10

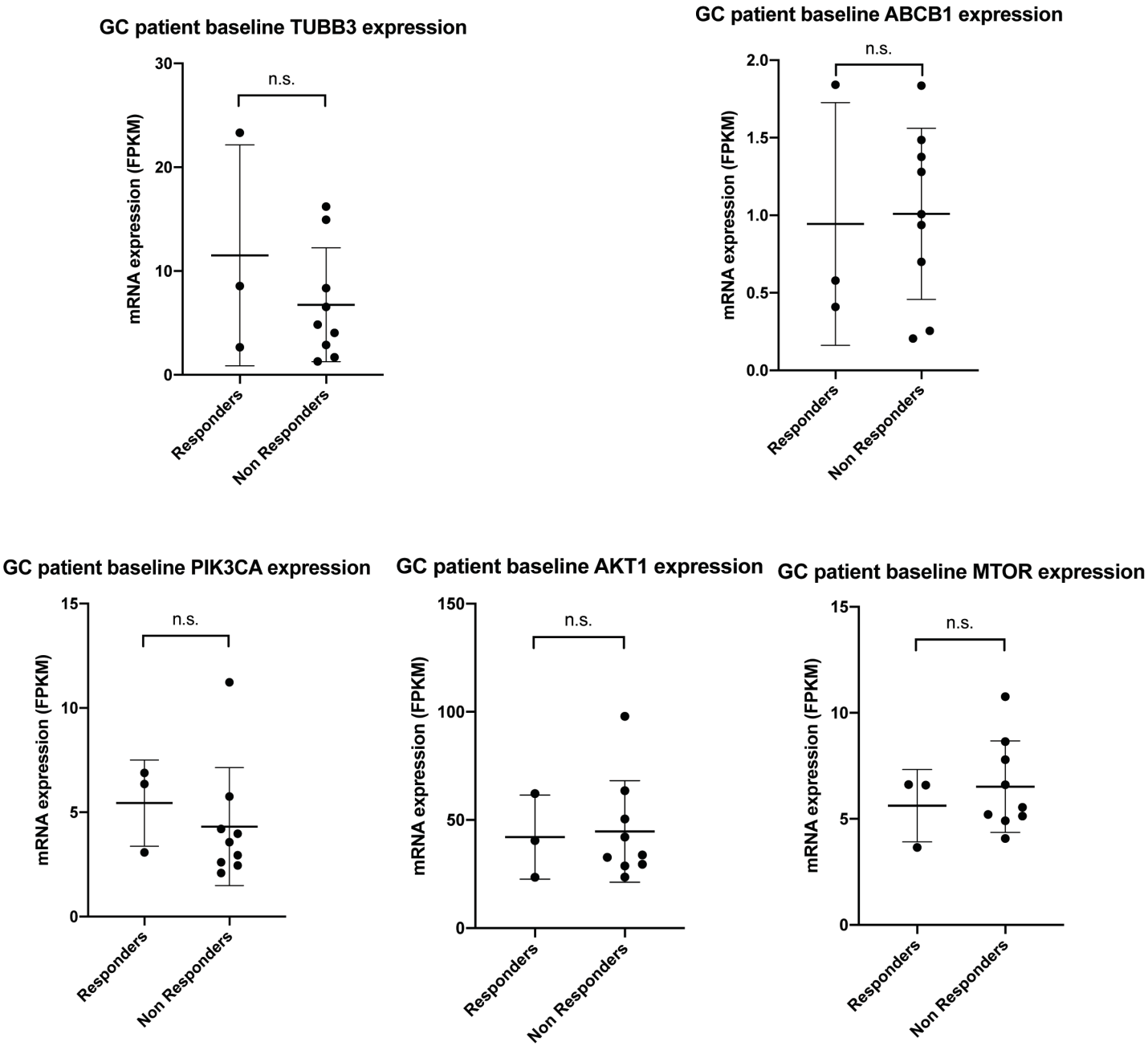


Figure S10. Baseline mRNA expression of the genes encoding for beta-III tubulin (TUBB3), P-glycoprotein (ABCB1) and the components of the PI3K intracellular signalling pathway in GC patients treated with taxane-treatment (cabazitaxel). Expression levels were correlated with clinical response. Mann-Whitney test was used for statistical analysis.

Table S1

	G-INTESTINAL				G-DIFFUSE							
	MKN-45	NCI-N87	KATO-III	AGS	SNU-1	TMK-1	MKN-1	AZ521	MKN-7	SCH	HS746T	FU97
DOCETAXEL IC50	0.2 nM	11 nM	8 nM	> 600 nM	0.2nM	0.08 nM	> 600 nM	13nM	> 600 nM	> 600 nM	>600 nM	> 600 nM
CISPLATIN IC50	12 uM	41 uM	48 uM	65 uM	4 uM	55 uM	19 uM	17 uM	25 uM	13 uM	120 uM	63 uM

Table S1. Cytotoxicity assay of docetaxel or cisplatin in GC cells. IC50 values
Mean IC50 values after 72-hour treatment with docetaxel or cisplatin in Intestinal and Diffuse GC cell lines. Values are calculated from 3 independent experiments. Cell lines with IC50 values exceeding the mean (relative resistant cells) are highlighted in red. Please, note the different sensitivity distribution between docetaxel and cisplatin, indicating the intrinsic and specific resistance to taxanes.